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(54) Title: METHODS AND MEANS FOR DETERMINING THE FEMALE FERTILE PERIOD

(57) Abstract

Provided is a highly reliable method to predict the beginning and/or ending of the fertile period for a female for each menstrual cycle. The methods and means provided advantageously address the day-to-day, cycle-to-cycle, and women-to-women variability in fertility hormone levels by analyzing the measurements of serial hormone concentrations in the midst of daily hormonal variations to determine when an actual and significant increase in the concentration of the monitored hormone has begun. Thus the present disclosure is directed to a method that combines existing hormone assay methods with calculation procedures to optimize the predictive values of daily hormonal changes. In this way a reliable and useful prediction of the fertile period is achieved with the concomitant assurance that the beginning and/or end of the fertile period has been reached so that such declarations are sufficient to provide both fertility enhancement and contraceptive utility.

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METHODS AND MEANS FOR DETERMINING THE FEMALE FERTILE PERIOD

Field of the Invention

The present invention relates generally to methods and means for determining periodic fertility of a female. More particularly, the present invention utilizes methods and means for determining when the fertile period begins via the evaluation of a series of measurements of urine hormones. The measurements are made with regular assay methods, essentially daily, after the first day of menses and continuing until the end of the fertile period. In addition, the present invention relates to a method for verifying the actual occurrence of ovulation, and therefore, the end of fertile period.

Background of the Invention

Methods for modifying human female fertility

There are several methods to modify human female fertility. Pharmacological agents can be utilized to modify either the endocrinological sequence or the viability of the gametes to reduce fertility. Examples are ovulation inducing drugs that enhance fertility and oral contraceptive and spermicides that reduce fertility. Mechanically, barriers such as condoms and diaphragms can be used during sexual intercourse to prevent unwanted pregnancy. Another widely practiced method in modifying fertility is the timing method. The timing method is based on analysis provides an estimate of when ovulation is likely to occur and thus sexual activity can be scheduled to maximize or minimize the probability of conception. Pharmacological and mechanical methods are beyond the scope of this invention as the present invention is directed to increasing the effectiveness of the timing method.

Timing ovulation

There are several known methods for approximating when ovulation has occurred, some of which are less reliable than others. Conventional methods for determining the fertile period, or for predicting the day of ovulation, range from tracking dates on a calendar to methods involving self-examination and the charting of body

temperature (sympto-thermal method).

The calendar method is based on the determination of the usual length of a woman's menstrual cycle and dividing by two. The presumption is that ovulation occurs approximately in the middle of the cycle. For example, if the woman's normal cycle length is 23-24 days, ovulation is presumed to occur on the 12th day; if the woman's normal cycle length is 40 days, ovulation may be presumed to occur between days 20 and 28. This method only provides a rough estimate of when ovulation may occur and is very difficult to use for most women whose cycle length varies from cycle to cycle.

Alternatively, a woman may know approximately when she is ovulating if she experiences a mid-month sharp pain in the lower abdomen, indicating that ovulation is about to, or has just occurred. However, this is not a very reliable sign as this pain can be confused with other pain and is not felt by every woman.

The sympto-thermal method increases the indicative value of the calendar method, and is based on the theory that basal body temperature rises discernibly (about 0.4° C) shortly after ovulation has occurred. This phenomenon is related to the production of progesterone by the Graffian follicle. Because ovulation usually occurs around the middle of the menstrual cycle, basal body temperature monitoring is begun at about that time. To detect the increase in temperature, a woman must take her temperature every morning, on waking, at the same time throughout the cycle and record the results, typically as a graph of temperature versus day of cycle. When an appropriately sensitive thermometer is properly used, a distinct increase in basal body temperature should be evident after ovulation has occurred. After recording this information over several months, a woman may be able to predict when ovulation will occur in subsequent cycles. However, as stated above, this method, is unsuitable for women who have irregular cycle length.

These methods of approximating day of ovulation have several disadvantages. A fundamental problem is the difficulty in predicting the length of the ongoing menstrual cycle from previous cycles because most women do not have a standardized cycle length

for ev ry cycle. When used as a method for preventing conception, each of these methods involves long intervals when users must practice abstinence or use additional contraceptive means. These methods may also involve ambiguity in interpreting the indicator of the beginning of the fertile period, as well as a high probability of method failure, i.e., conception. For example, the conventional sympto-thermal method of contraception involves an abstinence period or risk interval of ten days and has an efficacy (Pearl Index) of 22 pregnancies per 100 women-year of use. (Fertility & Sterility, 36: 152 [1981]; 36: 591 [1981]; 40: 773 [1983] and 44: 328 [1985].) Furthermore, the women in these studies were closely monitored, and therefore, the reported rate of pregnancy is probably the minimum rate to be expected when using the sympto-thermal method. The sympto-thermal method has an efficacy similar to the high end of the range of efficacy reported for users of barrier-type (condom, diaphragm) contraceptives.

Ovulation prediction by hormone assay

J.P. Royston (Biometrics, 38, 397-406, 1982) describes the relationship among basal body temperature, ovulation, and the risk of conception, with special reference to the lifetimes of sperm and egg.

A review article by the World Health Organization Task Force (Int. J. Fertil. 30(3), 18-30, 1985) describes a prospective multi-center study to develop universal immunochemical tests for predicting the fertile period in women.

Brown et al. (Am. J. Obstet. Gynecol. 157,1082-1089, 1987) describes a method for determining a woman's fertility period based on the measurement of estrogen and PDG in urine with an enzyme-immunoassay method.

Brown et. al. (Int. J. Gynecol. Obstet, Suppl. 1, 111-122, 1989) describes the importance of urinary estrogen and pregnanediol measurements in identifying the period of fertility and infertility during the menstrual cycle. They also describe an immunoassay based on reagents coated ont walls of a modified cuvette.

J.P. Royston (Statistics in Medicine, 10, 221-240, 1991) reviews rec nt

progress on the use of statistics in identifying the fertile phase of the human menstrual cycle. Methods using threshold, or Bayesian change-point, and CUSUM (cumulative sum) techniques are discussed.

Blackwell and Brown (Steroids, 57: 554-562, 1992) discuss immunochemical assay procedures and suggest to apply time series analysis for the recognition of increase in urinary estrogen as a marker for the beginning of the potential fertile period.

U.S. Patent No. 5,118,630 describes a method whereby an increase in pregnanediol glucuronide concentration in urine is compared to a threshold as a means to confirm ovulation.

WIPO Patent Publications WO 94/04924, WO 94/04925, WO 94/04926 and WO 94/04928 describe a method whereby hormones are measured, and along with information about the woman's prior cycles, are used to predict ovulation in the current cycle.

Recently, several methods have been made available to either predict the time of ovulation or to confirm its occurrence. These methods are typically based on changes in detectable levels of urine hormones. Urinary hormone concentrations vary throughout the menstrual cycle. For example, ovulation is generally preceded by an increase in urinary estrogen-type hormones. The level of luteinizing hormone ("LH"), increases significantly the day before ovulation occurs. In many tests, the increased LH concentration is indicated by a color change on a dipstick when contacted with a urine sample. Because the test result predicts ovulation before it has happened, it may provide a female with a better chance of conceiving as compared to the traditional methods that indicate that ovulation has already occurred. However, these ovulation prediction tests still have several disadvantages. When used for contraceptive purposes these tests must be performed very close to the time of ovulation since detectable LH increase occur only 24 hours before ovulation and LH levels return to normal in about 24 hours after ovulation. As stated above, it is not easy to predict the present cycle based on the history of previous cycles for women with irregular cycle lengths. A second drawback for the LH tests is that the strong surge in concentration happens too close to the

ovulation for contraceptive use. For contraceptive use, the LH test detects hormonal changes at a time too close to ovulation and therefore generally not sufficiently reliable. Furthermore, the LH surge does not always occur, rendering the determination of fertile period problematic.

As stated above, it is known that levels of progesterone are elevated shortly after ovulation, see e.g. U.S. Patent No. 5,118,630. A major disadvantage of this method is that it can only confirm ovulation. Therefore, it is of no use for either conception enhancement or contraceptive purposes, as it can only determine the post-ovulation infertile period, not the pre-ovulation infertile period. Again, the method relies on the previous cycle length to predict when ovulation is likely to occur so that a woman can start testing PDG levels. For these reasons, it would be beneficial to provide a method by which the beginning and/or end of the fertile period could be predicted reliably from hormone concentration measurements, without having to rely on unreliable timing methods or measurements.

Thus, there is a continuing need to provide a highly reliable method to predict the beginning, and/or the end, of a female's fertile period. In addition, a need exists to provide such a method which addresses the day-to-day, cycle-to-cycle, and woman-to-woman variability in fertility hormone levels. Furthermore, such a method should be preferably provided in a form and at a cost which is suitable for in-home use by an unskilled person.

The present invention provides a method that combines existing hormone assay methods with calculation procedures to optimize the predictive values of daily hormonal changes. In this way a reliable and useful prediction of the fertile period is achieved with the concomitant assurance that the beginning and/or end of the fertile period has been reached so that such declarations are sufficient to provide both fertility enhancement and contraceptive utility.

Summary of the Invention

In one aspect, the invention provides a method for determining the periodic fertile period in a female, the method including the steps of:

(a) measuring a hormone level in a daily sample of a biological fluid of the female for a testing period commencing on a fixed number of days from the start of menses;

b) storing the measured hormone level and corresponding day from start of menses into a data storage means;

(c) analyzing the hormone level using a computational means, the computational means comprising a microprocessor for applying a fitting function to normalized data;

(d) processing analyzed data in a regime of three stages, including a baseline stage, a predicting stage and a confirming stage;

(e) extrapolating an expected hormone level from the fitted function;

(f) the predicting stage comparing measured hormone level to extrapolated hormone level using a scoring means, the scoring means defining a preset relationship between measured and extrapolated hormone values;

(g) designating the first day of the fertile period as the day when accumulated scores in the predicting stage reach or exceed a predetermined value;

(h) the confirming stage storing the mesured hormone level from the first day of the fertile period until the end of the testing period;

(i) analyzing the stored hormone data at the end of the testing period with a second scoring means comprising a preset decision rule;

(j) designating the last day of the fertile period based on the outcome of the second scoring means; and

(k) displaying the first and last days of the fertile period on an output means; whereby the female is considered capable of conception from the time of reaching the first day of the fertile period until the last day of the fertile period.

The invention also provides a method for determining the first day of the fertile period.

The invention further provides a method for determining the final day of the fertile period.

The preferred method of the invention predicts both a first and a last day of the fertile period based on daily hormone measurements, most preferably EIG, commencing a fixed number of days usually 1 to 8, preferably 2 to 5, most preferably 5, from the start of menses. The daily hormone measurements continue for a fixed period of time, preferably 10 days, from the start of testing.

Brief Description of the Figures

Figure 1 shows a hard-held meter suitable for use in the method of the invention.

Figure 2 shows a disposable self-performing immunochromatography strip suitable for use in the method of the present invention.

Detailed Description of the Invention

The present invention relates to an improved method for predicting the day of ovulation by the prospective estimation of a future estrogen hormone level. Thus, in one aspect, the invention provides a method for monitoring an optimal period for conception. In addition, based on the prospective analysis of urinary progesterone hormone measurements, the present invention provides verification that ovulation has occurred. Therefore, in another aspect, by early prediction and verification of ovulation the invention provides a method for conception prevention.

Broadly, one method of the invention is begun by observing the first day of menses in the menstrual cycle. Starting from a fixed day of the menstrual cycle, preferably day 1 through day 8, the level of estrone is measured and the results recorded on a daily basis. Any number of measurement or assay methods known in the art may be used. However, an important criterion for choosing the measurement or assay method is that the measurement of the hormone marker should be at least precise enough to be semi-quantitative. The result is entered into a calculation means which stores the result and performs calculations, preferably on a daily basis, as will be described in

more detail below. The results of the first few days of hormone measurement thus serve as a baseline which is calculated for each female and, therefore reflects cycle variability unique to the user. When the progression of the change in estrone concentration meets the criteria set by the present invention, the beginning of fertile period is declared. From that day, the daily measurement of estrone ceases and a daily measurement of progesterone may begin. Again, progesterone may be measured following techniques known in the art as long as the method is suitable for at least semi-quantitative analysis. These results are also stored and analyzed by a calculation means. When the change in progesterone concentration meets the criteria set by the present invention, the end of the fertility period is declared.

Briefly, then, a method of the invention comprises the following elements:

a) daily measurements of the concentration of one or more fertility hormones in a biological sample;

b) a data storage means in which to input the hormone concentration data;

c) computational capacity for normalizing the data and fitting a function;

d) an evaluation means for extrapolating the fitted function to provide an estimate of the next day's hormone concentration;

e) a scoring means for decision making based on the relative slope of the fitted function and the comparison of the expected concentration to the observed concentration on the day of measurement; and

f) an output means to indicate the beginning of the fertile period or the end of the fertile period.

The result of the strip assay is read by the hand-held meter which also stores the result and performs calculations, as will be described in more detail below. The improved performance of the present invention over the other methods known in the art is due in part to the regime-like mathematical scheme tailored for this particular use which can reliably predict imminent ovulation. Essentially, the method utilizes a baseline region, a running window, a set of statistical decision making rules, and testing of a fixed duration. A baseline region is calculated and used to study the variation of the

observed hormone level in the present menstrual cycle itself as basis for later statistical decision. Each menstrual cycle uses its own baseline without the influence of or reference to previous menstrual cycles or other individuals. This simplifies operation and makes the method independent of cycle-to-cycle variability within the same individual. A running window scheme maximizes the efficiency and improves the sensitivity to significant changes in hormone levels. The statistical rules enhance the validity of decision making and the fixed duration of testing maximizes the efficiency of operation while minimizing expense and labor. The development and optimization of the mathematical scheme will be described in more detail below.

An alternate and preferred method of the invention begins by observing the first day of menses in the menstrual cycle. From a pre-selected fixed day, preferably day 5, the level of estrone is assayed and the results recorded on a daily basis. For assaying urinary steriod hormone, any number of assay methods known in the art may be used, but again, an important criterion for choosing the assay method is that the measurement of the hormone marker should be at least precise enough to be semi-quantitative. As before, the level of estrone in the first few days measurements serve as a baseline unique for the individual female. When an increase in hormone concentration meets the criteria described herein, the beginning of the fertile period is indicated to the user.

Again, any number of assay methods known in the art may be utilized in the method of the invention. In making the selection, an important criteria is that the assay provide at least a semi-quantitative result (as opposed to a qualitative, i.e., "yes/no" result) be "user-friendly" and uncomplicated. That is, the assay should be sutiable for in-home use by a relatively untrained person. Immunoassays developed for use on dip sticks, membranes, strips, etc. are thus preferred. Most preferred is a disposable, self-performing solid-phase immunoassay strip which, upon use, may be scanned by a reflectance reader as described below. The use of immunochromatography test stips is well known to those skilled in the art. Especially suitable for strips useful in the present invention are those employing colloidal microparticles. In this regard, the preferred microparticle is described in U.S. Patent 5,252,459 to Tarcha, et al. The '459 patent

describes an indicator reagent, method and test kit for the analysis of an analyte in a test sample.

As mentioned above, the end result of the hormone measurement must be normalized, fitted to a function to create a baseline against which subsequent hormone measurements will be compared. Such techniques can be performed manually following the method described infra, however, it is a preferred feature of the invention to utilize an essentially automated apparatus which performs all steps of the method which involve measurement, calculations, storage, etc. Thus, an additional and preferred feature of the invention is that the method of the present invention, when the calculation, storage, timing, etc. functions are handled by a microprocessor-based reader, requires no input, or interpretation of results, by the user. Indeed, when such a reader is utilized in the present invention, the information displayed by the reader is a signal or readout indicating that the fertile period has been reached. Thus, there is no quantitative or semi-quantitative result which is displayed for the user. This greatly simplifies the method from the standpoint of the user.

Thus, when the progression of the change in estrone concentration meets the criteria set by the present invention, the beginning of fertile period is declared. From that day, the daily measurement of estrone estrone continues until a pre-selected fixed day (e.g. day 14) for the end of monitoring. At the end of the monitoring period, the meter processes the stored results of the estrone level using the criteria set by the present invention to predict and declare the end of the fertility period. The improved performance of the present invention over the other methods known in the art is due in part to the invented regime-like mathematical scheme tailored for this particular use to predict imminent ovulation. Essentially, the mathematical scheme consists of a baseline region, a running window, a set of statistical decision making rules, and a fixed test duration. A baseline region is used to study the variation of the observed hormone level in the present menstrual cycle itself as basis for later statistical decision. Each menstrual cycle uses its own baseline without the influence of previous menstrual cycles or other individuals. This simplifies operation and avoids complication that no two cycles of

the same individual are identical. A running window scheme maximizes the efficiency and improves the sensitivity to detect a significant change in the hormone level. The statsitical rules enhance the validity of decision making. The fixed duration of testing maximizes the efficiency of operation and provide user with friendly operation system. The development and optimization of the mathematical scheme will be described in more detail below.

In a preferred method of practicing the invention, a hand-held meter is described for use in accordance with the invention. Figure 1 illustrates a hand-held meter suitable for use in fertility period monitoring. Figure 2 shows a strip for solid state immunoassay itself. Referring now to Figure 1, the meter 1 comprises a top casing 104 and a bottom casing 110 with an opening 109 for receiving the carrier 100. A test strip is held in place on carrier 100 by ridge 101 and locating pin 103. When placed in the meter, gear 102 of carrier 100 engages with the driving gear train of a motor inside the reader. An optical reflectance read head is housed inside the reader depicted as a protrusion 105 of the meter. Assay results as well as user inputs are displayed on a liquid crystal display 108. Two buttons or switches 106 and 107 provide control and user interface to the meter. Referring now to Figure 2, the solid phase immunoassay strip 2 is supported by a plastic carrier 200 which has a notch 201 complementary to locking pin 103 on carrier 100 to allow reproducible positioning. A reagent pad 202 contains, e.g. a diffusible labeled reagent, and is in contact with nitrocellulose strip 203 which contains an immobilized capture reagent for immuno reaction. When the user applies a urine sample to reagent pad 202, the reagent label reacts with steroid in the sample and the complex will be transported along the nitrocellulose strip by capillary action across the capture reagent which immobilizes the label-steroid complex. After an appropriate development time, the intensity of the signal at the capture reagent can then be recorded with a scanning reflectance reader (inside the meter under the read head) 105. Preferably, the meter is a battery powered device to enhance the portability and convenience of use of the whole system.

The hand-held meter is controlled by a microprocessor. Optical elements consist

of illumination and photo sensors which function to provide a data string as a reflection reading to a voltage-to-frequency converter for result signals to the processor. A motor provides scanning motion under control of the processor. A real time clock keeps track of timed parameters, e.g., day of testing and day of menstrual cycle. The user interface driver relays the information from and to the processor with the user through a display and control buttons. Batteries and a power supply controller ensures sufficient power that an entire menstrual cycle may be monitored before battery replacement is required. A calibration data bank is also included and functions to convert optical signal to analyte concentration.

As would be understood by the skilled person, individual features may be replaced by equivalent on substantially equivalent elements in order to achieve the same objective as outlined above. For example, discrete electronic parts could be replaced with an application specific integrated circuitry (ASIC) and visa versa.

Definitions

The following definitions are applicable to the present invention. Mathematical operations are also explained when appropriately associated with the definition.

The term "fitting function" refers to a mathematical expression of the relationship between the day of the menstrual cycle (typically, day 1 through 28) and the hormone concentration that is measured on that day. Although many fitting functions can be applied to the concentration data, three especially useful functions will be described which provide a high degree of certainty for the performance of the present invention.

a. Linear Function. The first fitting function is a simple linear equation and has the advantage of simplicity, fast calculation time, and easy implementation. The linear fitting function has the following formula:

$$\text{concentration} = a_0 + (a_1 \times \text{Day})$$

where a_0 is the intercept of the linear fitting function, a_1 is the slope of the linear fitting function and Day is the day of the menstrual cycle with Day=1 being the first day of

menses. a_0 and a_1 are numeric constants determined by the method of least squares. The unit of concentration in this equation is that used in the corresponding assay method. For example, nano-mole per liter is commonly used for assaying estrone 3-glucuronide (E1G) by immunoreaction. The linear least square procedure is easily calculated and is well known or can be readily found in any number of reference texts. e.g., "*FORTRAN Programs For Scientists and Engineers*", by Alan R. Miller (Sybex Inc., San Francisco (1982).

b. Linear Inverse Exponential Function. The second preferred function is a non-linear one and has the following formula:

$$\text{concentration} = (a_0 + (a_1 \times \text{Day}))/(1 + 1/10^{(a_2 + \text{Day})})$$

where a_0, a_1 and Day are as defined above, and a_2 is another numeric constant determined by the method of least squares. This non-linear fitting function is more complex since the exponential components require more computing power and time to execute. However, with its complex nature, it provides an opportunity to develop the adaptive algorithm to be discussed below. The numeric constants of the function are estimated by the method of least squares to provide a minimum squared difference between the concentration predicted by the fitting function and the actual concentration observed. For the non-linear fitting function, a_2 is the exponential modulus. The use of this function is more complex and usually involves multi-variable optimization iterative search. The Simplex method is one such well known method and is suitable for use in the method of the invention. A detailed description of the Simplex method can be found in standard linear programming problem solving texts such as *"Mathematical Programming"*, by Reinfeld and Vogel, Published by Prentice-Hall, Inc. Englewood Cliffs, N.J. (1958). Briefly, the Simplex method identifies a basic feasible solution for the constrained multi-variable equations. Then, the method is used to determine whether an optimum value has been reached by subtracting the value calculated from the fitting model for each basis point from the actual analytical value at the point. This difference

is squared and the squared difference is used to rank the sets of basis parameters. If an optimum has not been reached, the method provides a way of moving to a new solution that will have a better value of the objective function. In the method of the invention, for example, four observed hormone concentrations on four consecutive days are set up as four simultaneous equations with a set of starting estimated values of a_0, a_1, and a_2 with 4 slack variables, one for each of the four equations in a tableau. These variables are tested to see whether all the net gains are negative. If it is, then the optimum has been found. If not, the operation of pivoting is carried out using the most marginal net gain variable as the pivot element and removing the variable with the least positive net gain to arrive at a new basic feasible solution with modified variables. The process is repeated until the optimum has been found. The results are a set of values for the fitting function that give least square deviation for the observed hormone concentration.

c. Arithmetic Average Function. The third function is a simple arithmetic average calculation. By using average values from a selected range of days as a baseline to set up threshold values for comparison to the observed value of a later day, a mathematical relationship is set up. This function is the most simple to use but not very reliable if used to predict beginning of the fertile cycle because it is more sensitive to data variability than the previously described two functions. However, using a sophisticated function for prediction of the beginning of the fertile period to statistically compensate for the data variation difficulties, such as the linear equation or non-linear equation as described above, a simple method could be used for predicting the end of the fertile period.

The term "slope" refers to the rate of change in hormone concentration. For the linear fitting function, it is equivalent to the value of a_1. For the non-linear fitting function, it is the evaluation of the first derivative of the fitting function.

The term "relative slope" refers to the value of slope multiplied by 100 then divided by the average values of the observed hormone concentration in the fitting function. In other words, relative slope is the average concentration normalized rate of percentage concentration change. Since function fitting is done on the data in <u>base</u>

window, the slope is calculated from data in base window. [Define 'Base Window'] The use of relative slope instead of slope itself reduces sensitivity of decision making to the individual to individual variation.

The term "predicted value" refers to the threshold value of hormone concentration, based on the fitted function, extending one day beyond the fitting period. For example, if a four day fitting window is used the predicted value of today would the evaluation of the fitted function with the variables least square fitted from the observed hormone concentration of days from 4 days ago to yesterday.

The term "threshold value" refers to the value of the sum of predicted value and the value of fitting standard deviation multiplied by a chosen multiplier. By comparing the threshold value to the observed hormone concentration, the mathematical processes in the present invention determine whether a change in the trend in the hormone concentration has occurred. The multiplier is similar to a statistical 't' test and is selected using similar considerations in that the multiplier "assesses" the confidence level to be given an assessment that an observed change in hormonal concentration warrants an action. For the third fitting function of simple average as described above, the threshold value is the product of "baseline value" with a pre-set multiplier.

The term "data variability" refers to that property of the concentration measurements that reflect a female's own day to day hormone concentration changes and result in minor deviations from a smooth slope. For example, variability can be caused by the changes in the way the hormone is metabolized in liver, the rate of hormone secretion into the kidney, the volume of liquid secreted by kidney, the concentrating process of urine in the urinary bladder, and variations in the assay method used to quantify the hormone concentration.

The term "baseline" refers to the hormone concentrations as measured on several consecutive days that are characterized by relatively low values. Therefore, the baseline values correspond to days where an increase of ovarian activity is unlikely. Typically, baseline for E1G represents day 2 to day 5 of each cycle while the baseline for PDG represents the first 4 days of PDG measurement of each cycle.

The term "minimal average value" refers to the minimal value of a series of four day moving averages.

The term "fertile period" refers to that time of the menstrual cycle within which the presence of a viable sperm in the uterus, e.g, via sexual intercourse, would likely lead to conception.

The term "score" or "trigger point" refers to a number that is used to keep a record of an event. For example, the score is set to 0 when the menstrual cycle data analysis starts. When the observed assayed analyte concentration is higher than the threshold value and fulfills the decision rules as explained below, the score would be incremented by 1.

The term "decision rules" refers to the set of logical relationships that define decision making in the algorithm. This set of rules includes, but is not limited to the following: the difference between the observed concentration compared to the expected concentration; the relative slope compared to the preset threshold of relative slope value; the preset number of trigger points required for action compared to the accumulated trigger points of the cycle up to the day the decision is being made; and the maximal allowable fertile period. For example, a higher observed concentration than expected will give a trigger point if the slope is also positive. A trigger point will be given for each day having slope higher than threshold unless it is one of the baseline days. If the number of trigger points accumulated is equal to or greater than the number of trigger required for action, the beginning or end of the fertile period is declared and the output means will accordingly take action by displaying information and the computational and scoring means are prepared for the next phase, i.e., switching to accept an alternate assay result, e.g., PDG, if the beginning of the fertile period has been declared, or not accepting further assay results until the next cycle starts if the end of fertile period has been declared. For using the simple average function as described in the "fitting function" section above, there is no relative slope consideration in the decision rules. Instead, an additional rule is included that takes into account the number of days from the baseline calculation. As an example for this particular case, from the day after the

pre-set number of days from beginning of the baseline data of PDG assay, an observed value higher than threshold value would give a trigger point.

Homone Measurements

As stated above, hormone measurements may be made following techniques well known in the art. The assay method can use any of a variety of established biochemical or immunological procedures that can be used in either liquid format or in dry membrane bound solid phase format, such as chemical color reaction assay, enzyme-linked immunoassay (EIA), or radiolabeled immunoassay (RIA). For example, US Patent No. 4,138,278 describes a colorimetric immunoassay with enzyme label on solid surface for home pregnancy and ovulation tests, US Patent No. 5,120,643 describes a process for immunochromatography with colloidal particles, Japanese published application 1109262-A describes an immunoassay of estrogen hormones in body fluid having two or more antibodies to estrogen (conjugate), estradiol (conjugate), and/or estratriol (conjugate) immobilized on a solid phase, and Japanese patent application 88052705-B described measuring agglutination of latex reagents for diagnosis by measuring an increase in optical absorbance. For ease of use and convenience a solid phase format is preferred. The numerous methods and devices suitable for use in a solid phase format are well known in the art and no special mention need be made in this respect.

The hormones or hormone metabolites to be assayed may be obtained from any biological fluid in which such compounds are secreted by the female. Typically, blood and urine are preferred, an early morning sample of urine being the most preferred fluid. For instance, when the hormone estrodiol is measured, the assay can be estrone or other estrogen hormone in blood or the metabolites of the estrone hormones in urine such as estrone 3-glucuronide (E1G). U.S. Patent No. 3,544,868 provides a suitable method which can be used to determine steroid hormone glucuronide in urine samples by immunoassay with tracers of glucuronide bound to enzyme. In a similar manner, progesterone may be assayed using suitable markers such as serum progesterone or a

progesterone metabolite in urine samples such as pregnanediol-glucuronide (PDG). Again, any number of methods are known to those skilled in the art, such as gas chromatography, high performance liquid chromatography, enzymatic determination, immuno reactions, etc. and are suitable for use to practice the present invention.

The means by which the daily measurement of hormone concentration is accomplished are not important to the present invention. Any suitable means can be used including visual observation, spectrophotometric reading, radioactivity counting, or other electromagnetic readings as appropriate for the chosen assay method. When a solid phase format is used the measurement can be made most typically by a reader which detects and records results of electromagnetic readings. Readers appropriate for the chosen assay methods are well known and the above mentioned references illustrate the wide range of read out formats available. In a similar manner, the data storage means, computational capacity, evaluation means, scoring means, and output means can use any number of well known methods for accomplishing these functions. However, it is preferred to incorporate the above means into a single instrument that can function as a unitary device that would require essentially no input from the user other than to input a daily assay to be measured.

All references to patents or publications in this specification are incorporated herein by reference.

Examples

Example 1. Detection of the beginning of fertile period

In this example, the beginning of fertile period is predicted using a linear fit function and decision rules having the following parameters: observed hormone concentration greater than 2 times minimal average value for trigger point consideration, 6% relative slope is set as threshold after day 5, threshold value is set by adding 5 times standard deviation to the predicted value, and a score of 4 are required to declare action. As would be understood, this set of parameters is one of the many sets which may be used to assess the performance of the method of invention on a large data base. The particular parameters used here show the application of the method of the invention and

the resulting information which is provided by such data analysis. The optimum set of parameters for a large data base can be found by performing a designed experiment of systematic analysis using known ovulation day for reference verification. Further details of the optimization of parameters is found in Example 3 described below.

In this example the estrogen hormone estrone 3-glucuronide (E1G) is measured in urine. The procedure utilizes the World Health Organization radioimmuno assay (RIA) procedure. The first E1G assay is done on day 2 instead of day 1 so that a clear definition of the start of cycle is given and a simple daily routine is set up to facilitate sample collection and assay performance. The sample is collected from the first early morning passage of urine. Hormone assay is obtained for at least four days with results from each day entered into a microprocessor programmed for linear function calculation. The observed data of the first five days of the cycle are shown in Table 1.

The observations of Days 2-5 are fitted using the linear function and the various values described above are derived. In this case, the relative slope is not taken into account to increment the score because it is not after day 5 as stated in decision rules that relative slope factor would be considered only after day 5. Therefore, no adjustment is made to the score. The minimal average value is the same as average value observed because this four day period is the first window observed. Further, there is no predicted value calculation and the threshold value is set to a high number to indicate that no comparison is needed. On Day 6 the daily hormone measurement is made. The results are shown in Table 2.

The working window is shifted to day 2 to day 6 with a base window of day 2 to day 5. The observed day 6 value (89.35) is then analyzed according to the method of the invention. In this case the slope is positive (20.28) but the observed hormone assay value (89.35) is not higher than twice the minimal average value (76.35x(2)=153.7) and thus there is no change in the score. However, since the relative slope observed is higher than threshold slope (6) and the day is later than day 5 the score is incremented by 1. The score which results is less than set number of 3 and, therefore, day 6 is not declared as the beginning of the fertile period. The process of day 6 is repeated on day

7. The window shifts to Day 3-7 with a base window of day 3 to day 6. The data observed for day 7 and the processings are shown in the Table 3.

Since the observed value (120.37) is not higher than 2 times minimal average value (153.7), no score is incremented . Again, since relative slope (15) is higher than set threshold (6), the score is incremented by 1. The resulting score is not higher than that required for action and therefore Day 7 is not declared the beginning of the fertile period. This process is repeated until an action point is reached and the beginning of fertile period is declared. Table 4 shows the processing of data from other days.

On day 10, the score reaches the required number for action, namely 4. Thus the beginning of the fertile period for this cycle is declared on day 10. The ovulation day for this particular cycle had been independently established as day 13 by the daily ultrasound observation, i.e., day 13 showed characteristic reduction in follicle diameter as well as other features indicating ovulation such as echo observed in the follicle sac and blurring of follicle borders, etc. Therefore, in this example the method of the invention declares the beginning of fertile period 3 days prior to ovulation.

Example 2. Determination of the Ending of Fertile Period

This example describes the method of the invention for predicting the end of the fertile period. This example uses the same menstrual cycle as the one used in Example 1 and measures urinary pregnanediol 3-glucuronide (PDG) assay by the World Health Organization radioimmunoassay method described above.

Example 2(a)

This example uses the linear fitting function with the following parameters: 1 times minimal average value, 5 times standard deviation for threshold value, 12% relative slope, and a score of 3 for declaration of end of fertile period. Table 5 shows the observed concentration values and other parameters as analyzed for days 11 through 16 according to the method of the invention.

A four day baseline data is collected for initial relative slope and threshold value

calculation similar to the procedures described above for Example 1. In Exampl 2(a), day 11 to day 14 provides the four day baseline data of PDG. On day 15, PDG concentration observed, 11.54 uM, is higher than 1 times minimal average value and higher than threshold value of the day. Thus, the score is incremented by 1. The score is further incremented by 1 due to a relative slope higher (29.99) than threshold (12) in the base window. However, the accumulated score of 2 remains less than that required number for action and, therefore, this day is passed without declaring the end of fertile period. On day 16, observed value is greater than threshold value and score is thus incremented by 1. The resulting incremental score (3) is the number preset as required for action and day 16 is declared as the end of the fertile period for this particular cycle. This is three days after ovulation as determined by ultrasound (Example 1). No additional hormone assays are needed until the beginning of next cycle. At the first day of menses of the next cycle, the procedure for predicting the beginning of fertile period is carried out as described in Example 1.

Example 2(b)

This example uses the third fitting function of arithmetic averaging function. The parameters used are: 5 days after declaration of beginning of fertile period to begin fitting function for ending of fertile period, a value of 2 times the base value as the threshold value for ending of fertile period, and a score of 1 to declare action. In this case the multiplier for standard deviation is zero. The baseline data is obtained from day 11 to day 14. The arithmetic average method calculated the baseline value to 6.5 μM. The threshold value is then set to 13.0 μM (2 times base value; as set in the parameters). As shown in the Table 5, day 15 showed 11.54 μM which is lower than the threshold value of 13.0. Therefore day 15 is passed without action. On day 16, the observed PDG value is 13.28 μM, i.e., higher than threshold value. The score is incremented to 1 and the ending of the fertile period is declared because a score of 1 is pre-set for declaration of action. Again, the ending of the fertile period is three days post-ovulation as measured by ultrasound (Example 1).

Example 3. Finding the best set of parameters for a large data base

By collecting and analyzing a large database containing numerous data points, one can optimize the values assigned to the pre-set parameters, e.g., minimal average value, relative slope, threshold slope, multiplier, and the score. In this way the method of the invention can be customized to adjust for earlier detection and declaration of beginning of fertile period (for contraceptive utility) or to adjust for later (i.e., closer to ovulation) detection and declaration of beginning of fertile period (for conception enhancement utility).

For this purpose, a large data base with known ovulation day is required. A commonly practiced experimental design can be used for the optimization of parameters. Briefly, it involves many experimental runs with regularly varying combination of parameters. The trends of the effect of varying parameters are analyzed according to the desired relationship to the reference ovulation day. This analysis is then used to predict the best set of parameters for the desired relationship.

Tables 6-8 shows the results from a set of data base collected from 170 cycles. The time of ovulation of each menstrual cycle is determined by ultrasound investigation to within 24 hours certainty. Ovulation is declared when the sonographer notes both a reduction in follicle size and a change in follicle appearance by echo pattern and follicle border after rupture. The urinary E1G and urinary PDG assays are done following the RIA method of Examples 1 and 2. The parameters tested for this run on this data set are as follows:

For beginning of fertile period:

a minimal average value of 2.25 times background, 4.5% relative slope, a multiplier of 3.5 times standard deviation, and a score of 4 for declaration of action.

For end of fertile period:

6 days after declaration of beginning of fertile period to begin fitting function, 2 times minimal background value, 4.5 times background as threshold

value, a score of 1, and 11 days as maximal allowable fertile period.

Table 6 shows the results of predicting the beginning of fertile period of 170 cycles. The first column of the table lists the categories of results relating to ovulation day. For example -9 represents a result that beginning of fertile period is declared 9 days before ovulation occurred. The second column presents the number of cycles that are predicted to have the beginning of fertile period as the corresponding number of days from ovulation in the first column. The third column presents the number in the second column as a percentage fraction of the total number of 170 cycles. The fourth column represents the cumulated fraction of the cycles that have been predicted to have begun fertile period before and on the day shown in the first column before ovulation. The last column represents the remaining fraction of cycles that have not been announced to have entered fertile period. As can be seen in Table 6, a high majority of cycles (72%) are declared to have begun fertile period 3 days before ovulation and only a small fraction (5%) had not been declared to have entered fertile period by 1 day before ovulation.

Similarly, Table 7 shows the results of testing for the ending of fertile period of 170 cycles. The column and rows of data are arranged and defined as shown in Table 6. As can be seen in the cumulated fraction column, less than 5% of cycles are announced to have ended fertile period on and before the day of ovulation (as determined by ultrasound), while a majority of cycles (55% to 73%) are announced to have ended the fertile period by the time 4 to 5 days after ovulation.

As can be seen from these results, the predictive value of the method of the invention is superior to other methods known in the art.

Example 4. Comparison to known methods.

As an additional example, the results of the present invention are compared to those published by J.P. Royston, Statistics in Medicine, 10:221-240 (1991). The Royston reference summarized and assessed the performance of various predictive tests of fertile period based on published data. The data are incorporated into Table 8 and expanded to include data generated by the method of the invention. As is known in the

art (see, e.g., pp. 231 of Royston) a commonly accepted criteria for a critical fertility period includes the period of three days pre-ovulation and two days post-ovulation. In Table 8 parameter g is defined as the fraction of cycles that provided a fertile period that overlaps the critical period. Parameter T is an estimate of the dispersion of the distribution of the fertile period around the ideal value of 6. It is calculated by using the following formula:

$$T = \{ (f_a - 6)^2 + s_f^2 \}^{0.5}$$

where f_a stands for the average value of the fertile period and s_f stands for the standard deviation of the fertile period.

In Table 8, data from different sources are compared in terms of the average abstinence days and the standard deviation of the samples in their respective study in practicing each method. The g parameter calculated above using data generated following the method of the invention shows lower values than prior art methods for the reason that all the available data in the sample cycles were used in the calculation. For a practical use of the method, it would be more appropriate to include those cycles that did not give appropriate signals. Thus, when the cycles classified as not giving appropriate signals are also included into the calculation, the g values would about 0.61 to 0.64 for the chemical test 1 and chemical test 2 in Royston's table. If such g values are used, the values compare well with the results generated using the method of the invention (0.61). The smaller T value which results when using the method of the invention indicates that the present invention is capable of giving a much better defined fertile period in a more reproducible manner. Thus, one advantage of the present invention over the prior art methods of predicting fertile period is that a much shorter fertile period is assigned to each cycle.

Example 5. Trading optimization for a shorter predicted fertile period.

Examples 3 and 4 show a set of optimal parameters which provide good

performance and an acceptable predicted fertile period. This example shows that the parameters can be determined by following the experimental design used above but with the goal of a shortened predicted fertile period. This provides an additional advantage of fewer testing days which reduces the number of tests which must be taken and provides a more cost effective and easier to use method.

The optimization uses a 169 cycle data base from the same participants as used in Example 3, however, one of the 170 cycle data base was disqualified due to consecutive missed samples half way through the cycle. Ovulation of each menstrual cycle is determined by ultrasound investigation to within 24 hours certainty as before. However, in this case, the first urine sample collected for analysis is done on day 6 of menses. The urinary E1G and urinary PDG assays are done following the RIA method described above. The parameters are set as follows:

For beginning of fertile period:

Analysis begins on day 6 of menses, no minimal average value required above background, 4.5% relative slope, a multiplier of 3.5 times standard deviation, and a score of 3.

For end of fertile period:

No minimal average value required above background, 5% relative slope, a multiplier of 4 times standard deviation, a score of 4, and 10 days as maximal allowed fertile period.

Table 9 shows the results of predicting the beginning of fertile period of these 169 cycles. As can be seen in Table 9, a majority of cycles (59%) are declared to have begun fertile period 3 days before actual ovulation (as verified by ultrasound) and only a small fraction of cycles (3.6%) had not been declared to have entered fertile period by the day of ovulation.

Table 10 shows the results of testing for the ending of fertile period of the 169 cycles. As can be seen in the cumulated fraction column, less than 4% of cycles are predicted by the method to hav ended fertile period on or before th day of actual ovulation (as determined by ultrasound), while a majority of cycles (48% to 67%) are

predicted to have ended the fertile period by 4 to 5 days after actual ovulation.

A comparison of the results from Example 3 (the method of the invention commencing on day 2 of menses) and Example 5 (day 6) is shown in Table 11. As can be seen, the parameters assigned in Example 5 provide certain advantages over those used in Example 3. The predicted length of fertile phase is reduced from 8.4 to 8.1 days with a concomitant reduction in standard deviation of fertile phase from 2 to 1.5. However, this reduction in predicted fertile period results in a reduced index (g) of performance.

The effect of this reduction in g value on the predictive value of the method may be less than the numerical value would indicate since it appears that the reduction is predominately due to a difference of three vs two days pre-ovulation in predicting the start of fertile period. Furthermore, the lower g value may also be offset in part on the presumed use of an early morning urine sample. Therefore, the slightly lower g values which result from commencing the method of the invention on day 6 rather than day 2 are well compensated by the increased benefit of a shorter predicted fertile period and fewer testing days.

Example 6. Detection of the beginning of fertile period

In this example, the beginning of fertile period is predicted using a linear fit function and decision rules having the following parameters: 3% relative slope is set as threshold after day 5,expected value is set by adding 3 times standard deviation to the predicted value, and 3 trigger points are required to declare action. As would be understood, this set of parameters is one of the many sets which may be used to atssess the performance of the method of invention on a large data base. The particular parameters used here show the application of the method of the invention and the resulting information which is provided by such data analysis. The optimum set of parameters for a large data base can be found by performing a designed experiment of systematic analysis using known ovulation day for reference verification. Further details of the optimization of parameters is found in Example 8 described below.

In this example the estrogen hormone estrone 3-glucuronide (E1G) is measured in urine. The procedure utilizes a solid phase immunoassay on nitrocellulose. In this example in contrast to Example 1, the first E1G assay is done on day 5 instead of day 1 from the start of menses. The advantage of this most preferred method of the invention is that it reduces the number of assays per cycle required to be performed by the user as well as reducing the possibility of urine samples being contaminated by menstrual flow. Again, the sample is preferably collected from the first early morning passage of urine. Samples and assays are obtained and completed for the first four days (i.e., day 5 to day 8 of the menstrual cycle) with assay results from each day read and stored in the meter. The meter are programmed with the mathematical function of this invention. These first four days assay results serve as data the baseline data as explained above. As explained above, in the preferred method the meter is programed with function of the invention and thus the actual results are not viewed by the user. Accordingly, the internal operation of the calculation process inside the meter is described here in order to more fully explain the method of the invention. The observed data of a first four days results are shown in Table 12.

The observations of Days 5-8 are fitted using the linear function and the other related values described above are derived. Under the parameters set out above, the first four days serve as the baseline days, and, therefore, no trigger count adjustment is made. This results in the minimal average value being the same as the average value observed because this four day period is the first window observed. Furthermore, there is no predicted value calculation and the expected value is not applicable. On Day 9 a daily hormone measurement is made, the results of which are shown in Table 13.

The working window is now shifted to day 5 to day 9 with a base window of day 5 to day 8. The observed day 9 value (30) is then analyzed according to the method of the invention. In this case the slope is positive (14) but the observed hormone assay value (30) is not higher than the minimal expected value (67.9) and thus there is no trigger point for this feature. However, since the relative slope observed (14) is higher than threshold slope (3) and the day is later than day 8, the trigger point is incremented

by 1. However, the trigger point reached (1) is less than set number of 3 and, therefore, day 9 is not the beginning of the fertile period. The process of day 9 is repeated on day 10. The window then shifts to Day 6-10 with a base window of day 6 to day 9. The data observed for day 10 and the results of the method of the invention are shown in the Table 14.

In Table 14 it can be seen that the day 10 observed value (23) is not higher than the expected minimal average value (66.9), and this is no trigger points are incremented by this criterion. Again, since relative slope (17.6) is higher than set threshold (3), the trigger point is incremented by 1. The resulting trigger point reached (2) is not higher than trigger number required for action and therefore Day 10 is not declared the beginning of the fertile period. This process is repeated until an action point is reached and the beginning of fertile period is declared. Table 15 shows the processing of data on day 13 and the data from day from 9 to day 13 are displayed.

On day 13, the trigger point reaches the required number for action, namely 3. Thus the beginning of the fertile period for this cycle is declared on day 13. The ovulation day for this particular cycle had been independently established as day 17 by the daily ultrasound observation, i.e., day 17 showed characteristic reduction in follicle diameter as well as other features indicating ovulation such as echo observed in the follicle sac and blurring of follicle borders, etc. Therefore, in this example the method of the invention declares the beginning of fertile period 4 days prior to ovulation.

Example 7. Determination of the Ending of Fertile Period

This example describes the method of the invention for predicting the end of the fertile period and uses the same menstrual cycle as the one used in Example 6. Urinary estrone 3 glucuronide (E1G) is measured by the solid phase immunoassay as described above in Example 6. After the beginning of fertile period is declared in Example 6 day 13, the daily assay of urinary E1G continues until the pre-set day of day 14. On that day calculations commence using the same running window scheme of five days as that used for predicting the beginning of fertile period in Example 6, except the relative

slope is calculated from the day of observation itself. In this example the following decision rules are used: default fertile period is 9 days long, reduce fertile period by one day if maximum relative slope is greater than set threshold of 25%, extend fertile period by one day if the beginning of fertile period is less than 10 days while maximum relative slope is negative, reduce fertile period by 2 days if maximum relative slope reached was between day 11 and day 12, reduce fertile period by 1 day if the maximum relative slope is on day 13. Table 16 shows the observed concentration values and other parameters as analyzed for day 9 through 14 according to the method of the invention. As shown in Table 16, the relative slope (42.4%) observed on day 13, is greater than the relative slope of day 14 (13.0). According to the decision rules, a one day reduction infertile period is given for a relative slope greater than threshold (25%) and a fertile period is reduced by one day for reaching maximum on day 13. Therefore, the fertile period for this cycle is 7 days and the end of the fertile period for this cycle is calculated to be day 20. Ovulation day for this menstrual cycle had been determined by the ultrasound reference method on day 17 as mentioned above in Example 6. Thus, this declaration of end of fertile period provides 3 additional subsequent days to ovulation for contraception purposes.

Example 8. Determination of Optional Parameters

By collecting and analyzing a large database containing numerous data points, one can optimize the values assigned to the pre-set parameters, e.g., minimal average value, relative slope, threshold slope, multiplier, and the trigger number. In this way the method of the invention can be customized to adjust for earlier detection and declaration of beginning of fertile period (for contraceptive utility) or to adjust for later (i.e., closer to ovulation) detection and declaration of beginning of fertile period (for conception enhancement utility).

For this purpose, a large data base with known ovulation day is required. A commonly practiced experimental design can be used for the optimization of parameters. Briefly, it involves many experimental runs with regularly varying combination of

parameters. The trends of the effect of varying parameters are analyzed according to the desired relationship to the reference ovulation day. This analysis is then used to predict the best set of parameters for the desired relationship.

Tables 17-19 shows the results from a set of data base collected from 120 cycles. The time of ovulation of each menstrual cycle is determined by ultrasound investigation to within 24 hours certainty. Ovulation is declared when the sonographer notes both a reduction in follicle size and a change in follicle appearance by echo pattern and follicle border after rupture. The urinary E1G assay essentially follows the solid phase immunoassay as described in Examples 6 and 7. The parameters tested for this Example on this data set are as the same as described in Example 6 and 7.

Table 17 shows the results of predicting the beginning of fertile period for 120 cycles. The first column of the table lists the results obtained by the method of the invention as compared to actual ovulation day. For example -4 represents a result that the present method declares the beginning of fertile period 4 days before a confined ovulation had occurred. The second column presents the number of cycles that are predicted to have the beginning of fertile period as the corresponding number of days from ovulation in the first column. The third column presents the number in the second column as a percentage fraction of the total number of 120 cycles. The fourth column represents the cumulated fraction of the cycles that have been predicted to have begun fertile period before and on the day shown in the first column before ovulation. The last column represents the remaining fraction of cycles that have not been announced to have entered fertile period. As can be seen in Table 17, a high majority of cycles (66%) are declared to have begun fertile period 3 days before ovulation and only a small fraction (8%) had not been declared to have entered fertile period by 1 day before ovulation.

Similarly, Table 18 shows the results of testing for the ending of fertile period of 170 cycles. The data is arranged and defined as in Table 17. As can be seen in the cumulated fraction column, less than 8% of cycles are announced to have ended fertile period on and before the day of ovulation (as determined by ultrasound), while a majority of cycles (43% to 59%) are announced to have ended the fertile period by the

time 4 to 5 days after ovulation.

As can be seen from these results, the predictive value of the method of the invention is superior to other methods known in the art.

Example 9. Comparison to known methods.

As a further confirmation of the superior predictive use of the present invention, the results of the present invention are compared to those published by J.P. Royston, Statistics in Medicine, 10:221-240 (1991). The Royston reference summarizes and assesses the performance of various predictive tests of fertile period based on published data. The data are incorporated into Table 19 and expanded to include data generated by the method of the invention. As is known in the art (see, e.g., pp. 231 of Royston) a commonly accepted criteria for a critical fertility period includes the period of three days pre-ovulation and two days post-ovulation. In Table 19 parameter g is defined as the fraction of cycles that provided a fertile period that overlaps the critical period. Parameter T is an estimate of the dispersion of the distribution of the fertile period around the ideal value of 6. It is calculated by using the following formula:

$$T = \{ (f_a - 6)^2 + s_f^2 \}^{0.5}$$

where f_a stands for the average value of the fertile period and s_f stands for the standard deviation of the fertile period.

In Table 19, data from different sources are compared in terms of the average abstinence days and the standard deviation of the samples in their respective study in practicing each method. The g parameter calculated above using data generated following the method of the invention shows lower values than prior art methods for the reason that all the available data in the sample cycles were used in the calcualtion. For a practical use of the method, it would be more appropriate to include those cycles that did not give appropriate signals. Thus, when the cycles classified as not giving appropriate signals are also included into the calculation, the g values would approximately 0.65 to

0.68 for the either chemical test 1 or chemical test 2. If such g values are used, the values compare well with the results generated using the method of the present invention (0.68). The smaller T value which results when using the method of the invention indicates that the present invention is capable of giving a much better defined fertile period in a more reproducible manner. Thus, one advantage of the present invention over the prior art methods of predicting fertile period is that a much shorter fertile period is assigned to each cycle.

As can be seen from these results, the predictive value of the method of the invention is superior to other methods known in the art.

Examples 8 and 9 show a set of optimal parameters which provide good performance and an acceptable predicted fertile period. As is understood by those skilled in the art, the parameters can be determined by following the experimental design used above but with the goal of a shortened predicted fertile period. By applying the principle of design of experiments, another set of parameters could be devised to provide an additional advantage of fewer testing days which reduces the number of tests which must be taken and provides a more cost effective and easier to use method. On the other hand, another set of parameters, similar to Examples 8, could be devised to provide higher protection with the understood expense in running more assays for a longer period.

The alternative embodiments described are intended as examples rather than as limits. Thus, the description of the invention is not intended to limit the invention to the particular embodiments disclosed, but it is intended to encompass all equivalents and subject matter within the spirit and scope of the invention as observed above and as set forth in the following claims.

TABLE 1- Day 5

Cycle Day	Assay E1G (nM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
1							
2	62.61						
3	58.76						
4	75.33						
5	108.7	20.28		76.35	N/A	N/A	0

Note: N/A stands for not applicable.

TABLE 2- Day 6

Cycle Day	Assay E1G (nM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
2	62.61						
3	58.76						
4	75.33						
5	108.7	20.28	10.75	76.35	N/A	N/A	0
6	89.35	20.28	10.75	76.35	115.06	168.81	0

TABLE 3- Day 7

Cycle Day	Assay E1G (nM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
3	58.76						
4	75.33						
5	108.7	20.28	10.75	76.35	N/A	N/A	0
6	89.35	20.28	10.75	76.35	115.06	999999	1
7	120.37	15.07	13.71	76.35	114.32	168.81	2

TABLE 4- Day 10

Cycle Day	Assay E1G (nM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
6	89.35	20.28	10.75	76.35	115.06	999999	1
7	120.37	15.07	13.71	76.35	114.32	168.81	2
8	99.03	11.76	13.33	76.35	127.38	182.89	3
9	162.64	0.19	13.27	76.35	104.87	194.01	3
10	182.20	10.83	19.36	76.35	167.48	171.22	4

TABLE 5- Day 16

Cycle Day	Assay PDG (uM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
11	2.77						
12	5.25						
13	5.39						
14	12.6	45.57					0
15	11.54	45.57	1.83	6.50	13.91	8.23	2
16	13.28	29.99	2.01	6.50	15.21	23.05	3

TABLE 6

Days from ovulation Day	No. Cycle predicted to begin Fertile period	Fraction (%) of total 170 cycles	Cumulated Fraction (%)	Remaining Fractions (%)
≤ -9	15	8.8	8.8	91.2
-8	6	3.5	12.3	87.7
-7	9	5.3	17.6	82.4
-6	11	6.5	24.1	75.9
-5	26	15.3	39.4	60.6
-4	29	17.1	56.5	43.5
-3	27	15.9	72.4	27.6
-2	20	11.8	84.2	15.8
-1	18	10.6	94.8	5.2
0	7	4.1	98.9	1.1
1	1	0.6	99.5	0.5
≥ 2	1	0.6	100.0	0.0

TABLE 7

Days from ovulation Day	No. Cycle predicted to end Fertile period	Fraction (%) of total 170 cycles	Cumulated Fraction (%)	Remaining Fractions (%)
≤ -2	1	0.6	0.6	99.4
-1	2	1.2	1.8	98.2
0	5	2.9	4.7	95.3
1	11	6.5	11.2	88.8
2	22	12.9	24.1	75.9
3	27	15.9	40.0	60.0
4	25	14.7	54.7	45.3
5	31	18.2	72.9	27.1
6	21	12.4	85.3	14.7
7	9	5.3	90.6	9.4
8	7	4.1	94.7	5.3
≥9	9	5.3	100.0	0.0

TABLE 12- Day 5 to Day 8 Baseline Region Data

Cycle Day	Assay E1G (nM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
5	32						
6	15						
7	33						
8	40	4.2	9.13	30	N/A	N/A	0

Note: N/A stands for not applicable.

TABLE 13- Day 9

Cycle Day	Assay E1G (nM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
5	32						
6	15						
7	33						
8	40	14	9.13	30	N/A	N/A	0
9	30	14	9.13	30	40.5	67.9	1

TABLE 14- Day 10

Cycle Day	Assay E1G (nM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
6	15						
7	33						
8	40	14	9.1	30	N/A	N/A	0
9	30	14	9.1	30	40.5	67.9	1
10	23	17.6	8.1	29.5	42.5	66.9	2

TABLE 15- Day 13

Cycle Day	Assay E1G (nM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
9	30	14	9.1	29.5	40.5	67.9	1
10	23	17.6	8.1	29.5	42.5	66.9	2
11	37	-12.7	4.8	29.5	21.5	999999	2
12	84	-4.9	7.3	29.5	28.5	999999	2
13	90	40.5	15.7	29.5	87.5	134.6	3

TABLE 16- Day 13 and Day 14

Cycle Day	Assay PDG (uM)	Relative Slope (%)	Standard Deviation	Minimal Average Value	Predicted Value	Expected Value	Trigger Points
13	90	42.4	15.7	29.5	87.5	134.6	3
14	65	13.0	2.01	6.50	15.21	23.05	3

TABLE 17

Days from confirmed ovulation (1*)	No. Cycle predicted to begin Fertile period	Fraction (%) of total 120 cycles	Cumulated Fraction (%)	Remaining Fractions (%)
≥ -9	7	5.8	5.8	94.2
-8	5	4.2	10.0	90.0
-7	2	1.7	11.7	88.3
-6	7	5.8	17.5	82.5
-5	21	17.5	35.0	65.0
-4	14	11.7	46.7	53.3
-3	23	19.2	65.8	34.2
-2	16	13.3	79.2	20.8
-1	15	12.5	91.7	8.3
0	7	5.8	97.5	2.5
1	2	1.7	99.2	0.8
≥ 2	1	0.8	100.0	0.0

1* = Confirmed by ultrasound, see text.

TABLE 18

Days from confirmed ovulation (1*)	No. Cycle predicted to end Fertile period	Fraction (%) of total 120 cycles	Cumulated Fraction (%)	Remaining Fractions (%)
≤ -2	3	2.5	2.5	97.5
-1	3	2.5	5.0	95.0
0	4	3.3	8.3	91.7
1	3	2.5	10.8	89.2
2	4	3.3	14.2	85.8
3	11	9.2	23.3	76.7
4	23	19.2	42.5	57.5
5	20	16.7	59.2	40.8
6	18	15.0	74.2	25.8
7	15	12.5	86.7	13.3
8	10	8.3	95.0	5.0
≥ 9	6	5.0	100.0	0.0

1* = Confirmed by ultrasound, see text.

TABLE 19

Method	Length of fertile phase		Indices of performance	
	Mean (days)	SD (days)	g	T
Sympto-thermal	13.4	2.9	0.98	7.9
Cervical Mucus (billings)	11.9	2.9	0.91	6.6
Thermal (Calculation and BBT)	11.8	3.3	0.90	6.7
Chemical test 1	9.3	2.2	0.83	4.0
Chemical test 2	10.7	2.3	0.84	5.2
This invention	8.4	2.0	0.65[a]	2.7
This invention	8.4	2.0	0.68[b]	2.7

Note: a: calculated with all cycles using -3 and +3 from ovulation as criterion.
b: calculated with all cycles using -3 and +2 from ovulation as criterion.

We Claim:

1. A method for determining the periodic fertile period in a female, the method comprising the steps of:

(a) collecting a daily sample of biological fluid from the female commencing start of a fixed number of days from the menses and measuring a hormone level in the daily sample for a number of consecutive days;

(b) inputting the measured hormone level and corresponding day from the first day of menses into a data storage means;

(c) analyzing the hormone level using a computational means, the computational means comprising the application of a fitting function, extrapolating a threshold value of hormone level from the fitted function, comparing the measured hormone level and the threshold value using a scoring means, the scoring means defining preset relationship between the measured hormone level and threshold value;

(d) defining a first day of the fertile period and a final day of the fertile period as the day when application of the scoring means results in an accumulated score greater than a preset value; and

(e) displaying a predicted first day of the fertile period and a predicted final day of the fertile period on an output means;

whereby the female is considered capable of conception from the time of reaching the first day of fertile period until the final day of the fertile period.

2. A method of claim 1 further comprising the measurement of a first hormone for a first period commencing a fixed number of days from the start of menses and a second hormone for a second period commencing the end of the first period.

3. The method of claim 2 wherein the first hormone is estrogen or an estrogen metabolite and the second hormone is progesterone or progesterone metabolite.

4. The method of claim 3 wherein collecting the first period commences the second day of menses.

5. The method of claim 3 wherein collecting the first period commences the sixth day of menses.

6. A method for determining the periodic fertile period in a female, the method comprising the steps of:

(a) measuring a hormone level in a daily sample of a biological fluid of the female for a testing period commencing on a fixed number of days from the start of menses;

b) storing the measured hormone level and corresponding day from start of menses into a data storage means;

(c) analyzing the hormone level using a computational means, the computational means comprising a microprocessor for applying a fitting function to normalized data;

(d) processing analyzed data in a regime of three stages, including a baseline stage, a predicting stage and a confirming stage;

(e) extrapolating an expected hormone level from the fitted function;

(f) the predicting stage comparing measured hormone level to extrapolated hormone level using a scoring means, the scoring means defining a preset relationship between measured and extrapolated hormone values;

(g) designating the first day of the fertile period as the day when accumulated

scores in the predicting stage reach or exceed a predetermined value;

(h) the confirming stage storing the measured hormone level from the first day of the fertile period until the end of the testing period;

(i) analyzing the stored hormone data at the end of the testing period with a second scoring means comprising a preset decision rule;

(j) designating the last day of the fertile period based on the outcome of the second scoring means; and

(k) displaying the first and last days of the fertile period on an output means; whereby the female is considered capable of conception from the time of reaching the first day of the fertile period until the last day of the fertile period.

7. A method for determining the beginning of the periodic fertile period in a female, the method comprising the steps of:

(a) collecting a daily sample of biological fluid from the female commencing on a pre-selected fixed day after start of menses and continuing through a designated first day of the fertile period;

(b) measuring estrogen-related hormone level in the sample;

(c) storing the measured hormone level and corresponding day from start of menses on which the hormone level is measured into a data storage cans;

(d) analyzing the hormone level using a computational means, the computational means composing a microprocessor for applying a fitting function to normalized data; such that the computational means calculates a trend in hormone level variations and determines expected hormone level levels based on a trend in measured hormone level variations;

(e) comparing measured and extrapolated hormone levels using a scoring means;

(f) designating the first day of said fertile period when application of the scoring means results in an accumulated score greater than a preset value; and

(g) displaying a result indicating that the female is considered capable of conception after the first day of the fertile period.

8. A method for determining the end of the periodic fertile period in a female, the method comprising the steps of:

(a) collecting a daily sample of a biological fluid from the female commencing on a fixed day of the female menstrual period and continuing for a fixed number of days of menstrual period;

(b) measuring an estrone-related hormone level in the sample;

(c) storing the estrone-related hormone level and corresponding day from start of menses on which the hormone level was measured into a means for computing a trend in daily variations in measured hormone levels;

(d) scoring variations in daily measured estrone-related hormone levels using a scoring means; and

(f) designating the final day of the fertile period when the scoring means results in an outcome of a preset decision rules; whereby the female is considered capable of conception from the time of reaching the first day of fertile period until the final day of the fertile period.

9. A method according to claim 6 wherein the testing period commences on day 5.

10. A method according to claim 6 wherein the testing period commences on day 2.

11. A method according to claim 6 wherein the baseline is 4 days.

12. A method according to claim 6, wherein the testing period is 10 days.

13. A method according to claim 6 wherein the hormone is estrone 3-glucuronide (E1G).

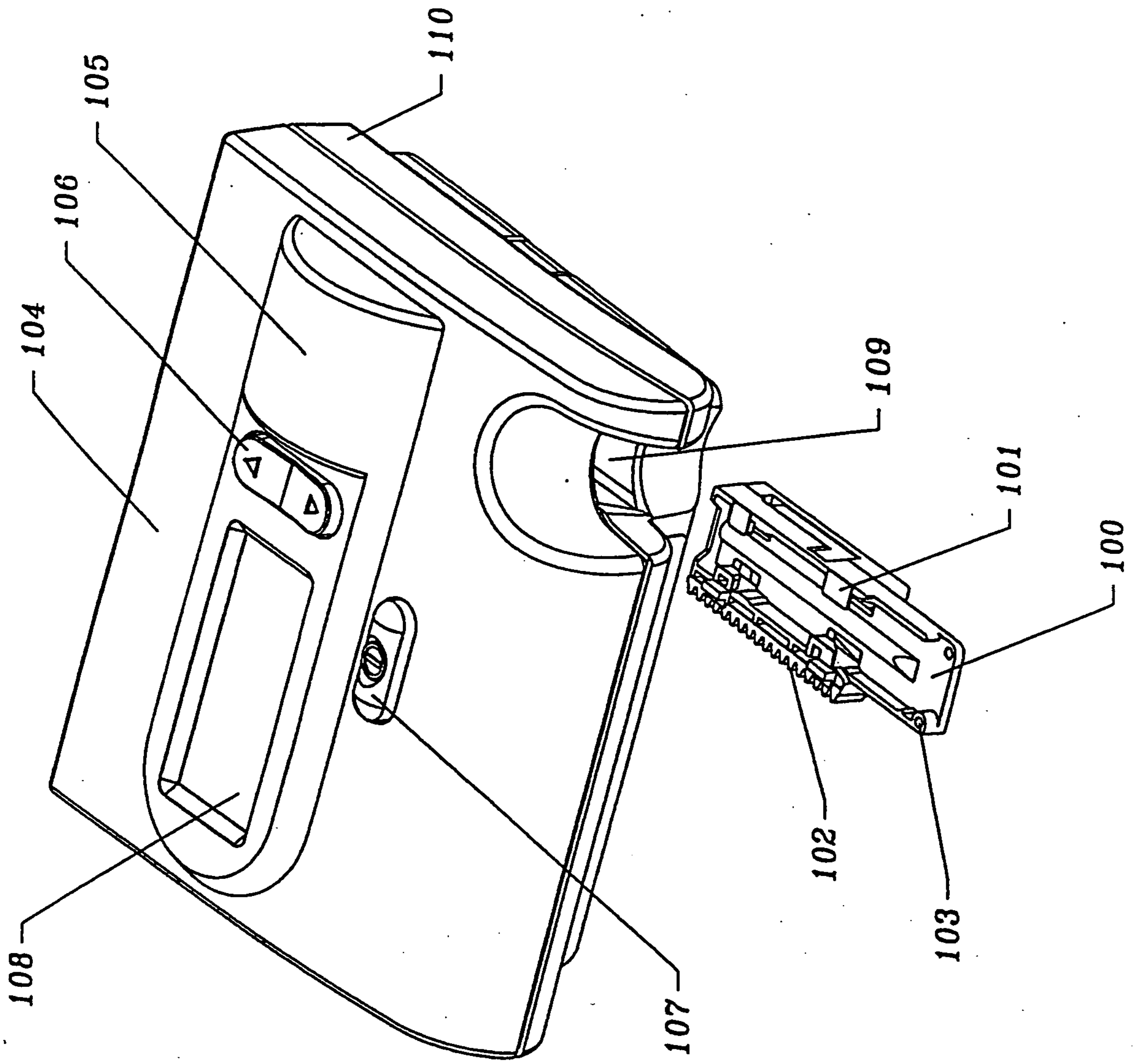

FIG. 1

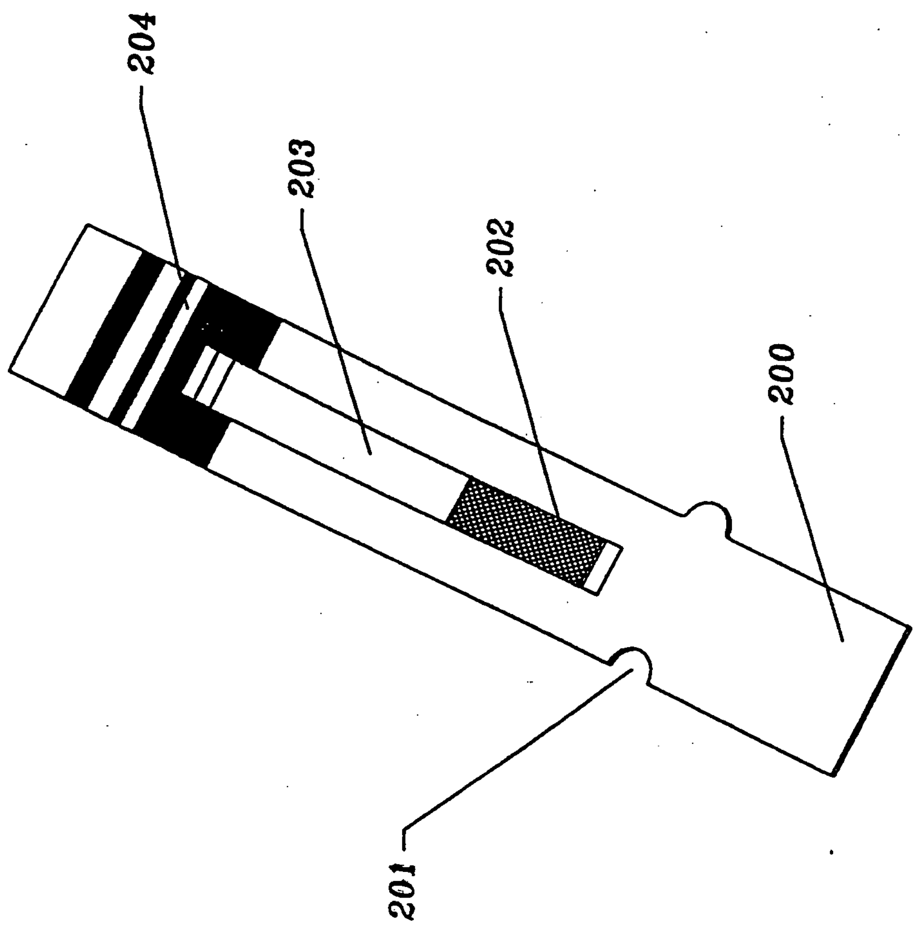

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/14455

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/74 G06F15/42
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N G06F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 367 615 (MONOCLONAL ANTIBODIES, INC.) 9 May 1990 see the whole document ---	1-13
Y	EP,A,0 086 095 (T. S. BAKER ET AL.) 17 August 1983 see the whole document ---	1-13
Y	GB,A,2 029 011 (T. S. BAKER ET AL.) 12 March 1980 see the whole document ---	1-13

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 May 1995	17.05.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Griffith, G

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International Application No
PCT/US 94/14455

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	WO,A,93 07578 (E. J. BIGGEL.) 15 April 1993 see the whole document	1-13
Y	DE,A,31 12 767 (DR. W. STEINSCHULTE.) 11 November 1982 see the whole document	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 94/14455

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DE-A-3112767	11-11-82	NONE		